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BIOLOGICAL BULLETIN

CARBON DIOXIDE PRODUCTION IN RELATION TO REGENERATION IN PLANARIA DOROTOCEPHALA.¹

HARRIET L. ROBBINS AND C. M. CHILD.

Numerous lines of evidence indicate that the body of *Planaria dorotocephala* consists physiologically of more than one individual or "zooid" after a certain limit of size is exceeded in the course of growth. The first or chief zooid of the series includes the region from the head to a level slightly posterior to the mouth, the level at which fission usually occurs, and the region posterior to this consists of one or more short zooids, the limits of which can be distinguished physiologically, but not morphologically (Child, '10, 11c, '15, chap. VI). The susceptibility of the body to a large number of chemical and physical agents in concentrations or intensities too high to permit acclimation or tolerance, decreases from the head posteriorly through the length of the first zooid, increases sharply at the level of fission and in long animals shows one or more rises posterior to that level (Child '13b). The process of regeneration also shows graded differences at different levels, corresponding to the differences in susceptibility (Child '11b).

In the papers referred to, as well as in many others evidence has been presented to that the susceptibility differences in general are in some degree an indicator of quantitative differences in metabolic condition, particularly in rate of oxidations, though susceptibility has never been regarded as an exact quantitative measure of oxidation. According to this point of view the gradations of susceptibility in the body of *Planaria dorotocephala* indicate that the rate of oxidation decreases gradually, at

¹ From the Hull Zoölogical Laboratory, University of Chicago.

least in the ectoderm and body wall, from the head to the level of fission and rises suddenly at the anterior end of the second zooid and of each further zooid, if such are present.

It has also been shown that in isolated pieces of the body of *Planaria*, particularly in those below a certain fraction of the body length, susceptibility is increased during the first few hours following section. This temporary increase of susceptibility, apparently associated with the stimulation of the pieces by section, is least in pieces nearest the head, and increases in successive pieces posteriorly to the level of fission, where it again decreases (Child, '14). In general the regions of highest susceptibility in the intact animal show the least increase in susceptibility in isolated pieces, and vice versa. Within twelve to twenty-four hours after section the temporary increase in susceptibility has disappeared and the susceptibility of each piece is about the same as, or slightly lower than before section. From twenty-four to forty-eight hours after section a gradual rise in susceptibility begins as the regulatory processes leading to the development of a new individual make themselves evident, and the susceptibility after regeneration is completed is much higher than that of the region of the body of the original animal which a given piece represents. This is true not only for the newly developed head and posterior end of the regenerated animal but for the whole body (Child, '15, Chap. V). In the light of various facts, these changes in susceptibility have been interpreted as indicating and in some way associated with changes in rate of oxidation. The present paper constitutes additional evidence for this conclusion. Carbon dioxide production was chosen instead of oxygen consumption as the subject of investigation first because investigation of the temporary changes, immediately following section involves the technical difficulty of preparing very large numbers of pieces within a very short time in order that oxygen consumption may be sufficient for determination in short periods, and second because work on oxygen consumption during the course of regeneration was already in progress in this laboratory. The colorimetric method was used since it is well adapted for obtaining comparative data with small amounts of material.

A few experiments similar to those recorded below were performed by Child from time to time during several years past, and these experiments, although they brought to light certain difficulties as regards technique and were too few in number to constitute conclusive evidence, indicated very clearly that the differences and changes in susceptibility following section and after regeneration are paralleled by differences and changes in rate of carbon dioxide production. A more extensive investigation of the subject by another person was desirable, and this was undertaken by Miss H. L. Robbins in candidacy for the degree of M.S. The data tabulated in the present paper are those obtained in this investigation.

The special technique for the different experiments is described in the following sections, but the general technique used in connection with the colorimetric method is very similar to that employed in the study of CO₂ production during starvation (Child, '19a). The weighing of the animals or pieces is the most difficult feature of the preparation, since the presence of superfluous water introduces an error in weight, and since weighing must be done as rapidly as possible to avoid injury or death from drying. After considerable practice, involving repeated weighings of the same lots and determination of the length of time which could be allowed without injury for drainage on filter paper and exposure to the air while weighing, a satisfactory method of procedure was developed, and this was followed in all the tabulated experiments. The container was first weighed and both container and weights were left on the balance pans: next weights equal to the estimated weight of the lot of worms or pieces were placed on the weight pan in order to reduce the time necessary for weighing the animals: the worms were then brought together at the tip of a funnel of well washed filter paper, drained for a certain length of time, transferred on a slightly vaselined scalpel blade to the container and weighed, each of these operations being performed in as nearly as possible the same length of time in each case. Equal or approximately equal weights of the lots to be compared were used (see tables). For the colorimetric estimations the Hynson Westcott and Dunning *H* ion outfit with phenolsulphonephthalein as indicator

was used. After weighing, the worms were returned at once to water and then transferred to pyrex tubes fused at one end and of the same diameter as the standard tubes. After washing twice in aqueous indicator solution of the same concentration as that in the standard tubes, each tube was filled with indicator solution to a 3-c.c. level previously marked. The tubes were then sealed without air bubbles by running in on the surface of the fluid about 1 c.c. of soft paraffin at a temperature just above melting point, the worms being kept at the bottom of the tube to avoid injury from change of temperature. Since leakage past the paraffin plug is difficult to avoid when changes of temperature occur and since it was found desirable to reduce the temperature slightly as a means of keeping the animals quiet (see below), the following method of providing for changes in volume of the fluid was used. A piece of closely fitting soft rubber tubing, previously coated with soft paraffin was drawn over the open end of the pyrex tube, leaving 2 to 3 cm. of the tubing beyond the end of the glass. Indicator solution was then added to fill both the glass tube above the paraffin plug and the rubber tube, and the latter is then closed by a screw clasp. This procedure makes impossible the entrance of air past the paraffin plug when the temperature is lowered. Instead of air a small amount of the indicator solution above the plug may be drawn below, but this occurs within the first five minutes or less of the experiment, and with the changes of temperature involved the amount of fluid passing the plug is negligible, so far as the results are concerned. The indicator solution between the paraffin plug and the rubber tubing is visible and serves as a control for the color change below the plug. Closure by means of the rubber tubing and clamp alone was found to be unsatisfactory because some of the worms creep into the rubber tubing, where they cannot be seen, and it is therefore impossible to determine whether motor activity is going on and whether all are in good condition. Moreover, pieces in the rubber tubing are often overlooked when the lots are removed from the experimental tube and the whole lot becomes valueless for further experiment, unless substitution for the lost pieces is made, but this is at best an undesirable procedure.

The *pH* at the beginning of the experiment was of course the same in all lots to be compared, but the starting point differed somewhat in different experiments, the extreme range being 7.8 to 7.95. Observations were made at least every half hour with few exceptions, but the tables, instead of recording all the *pH* readings, give the times required to reach an arbitrary end point, *Hp* 7.3 being selected as this end point. As each lot approached this point, observation was more or less continuous. A daylight lamp was used for all color comparisons.

A serious difficulty in the earlier experiments, particularly with the pieces after section, was the occurrence of motor activity, which of course increased CO₂ production and introduced a source of error. In these experiments as in earlier work, it was observed that pieces from regions near the head are much more likely to show apparently spontaneous motor activity during the first few hours after section, than pieces from the more posterior levels of the first zoid. After attempting in various ways to eliminate motor activity, it was found that decrease of a few degrees in temperature was usually effective for the length of time necessary. In all the experiments tabulated below the animals were kept at 21° to 22° C in the stocks and during preparation, but as soon as the tubes were sealed they were placed in water at a constant temperature of 18° in very dim diffuse daylight. Under these conditions motor activity occurred only rarely, but its occurrence was always noted in the record of the experiment. The procedure adopted in these experiments has been described at some length because in experimental work with animals of such small size it is extremely easy to go astray, if the various sources of error are not carefully controlled as far as possible.

THE STIMULATION OF PIECES FOLLOWING SECTION.

The temporary increase in susceptibility following section and its characteristic relation, both to size of piece and region of body (Child, 14, also p. 104 above) are so clearly shown by the susceptibility method that they are often used as laboratory experiments. Because they are temporary and apparently excitatory in character and so definitely related to size of piece

and region of body these changes in susceptibility are of special interest in connection with the question of the relation between susceptibility and metabolism. If changes in the rate of fundamental metabolism or of certain fundamental reactions are found to parallel these changes in susceptibility, it is evident that the susceptibility method, when properly used, is a rather delicate indicator of at least certain aspects of metabolic condition.

In the earlier experiments of Child, as well as in the preliminary work of Robbins, it was found that the changes and differences in CO_2 production following section appeared more clearly in animals which had been starved for a few days before experiment, than in those which had been more recently fed, although in the former the total CO_2 production and oxygen consumption are less than in the latter (Child, '19a, Hyman, '19b). It has been pointed out elsewhere (Child, '19b, '19c and various earlier papers), that the susceptibility method as used in these experiments gives information primarily concerning conditions in ectoderm and body wall, though with certain precautions it may be used to show differences in condition in the alimentary tract of *Planaria* and other forms. The susceptibility data, as well as other facts, indicate that the changes following section are at least in large measure confined to ectoderm and body wall, the alimentary tract not being affected to any great degree. The CO_2 production of the alimentary tract in fed animals constitutes, however, a large proportion of the total CO_2 production, moreover, the volume of the alimentary tract as compared with that of other organs is greater and a larger amount of food and reserves is usually present in regions near the mouth than in regions near the head, therefore it is desirable to decrease this alimentary CO_2 production as far as possible, in order that changes in other parts of the body may appear more clearly. It has been shown (Child, '19a, Hyman, '19b) that a rapid decrease in both CO_2 production and oxygen consumption occurs in *Planaria dorotocephala* during the first few days of starvation and that an increase in both follows so rapidly after even a single feeding that it cannot be due to oxidation of the food following assimilation, but must be due to stimulation of the alimentary tract by the food. Allen ('19) has recently recorded the occurrence of similar

changes in oxygen consumption in two other species of *Planaria* during the early stages of starvation. It is evident that the rapid decrease in CO₂ production and oxygen consumption during the early stages of starvation is due in large measure, if not wholly, to the decrease in functional activity of the alimentary tract in the absence of food newly ingested. In the light of all these facts the reason for the use in these experiments of animals which have been starved a few days is evident. The length of the period without food is given in each experiment: in no case is it long enough to produce any marked reduction in size or other changes except those in the alimentary tract.

Since the purpose of these experiments is to determine whether differences and changes in susceptibility following section are paralleled by differences and changes in CO₂ production, the size of animals and pieces used and the regions of body included are those which show the most definite and characteristic differences and changes in susceptibility. The experimental material for the data presented in Table I. was prepared as follows: animals sixteen to eighteen mm. were selected from well fed laboratory stock, those which had recently undergone fission being excluded, were kept without food for several days (see Table I.) and were then cut into pieces as indicated in Fig. 1, piece *C* being cut so that the greater part of the pharynx is separated from its attachment and is extracted from the pharyngeal pouch, *i.e.*, piece *C* contains a part of the pharyngeal pouch, but no portion of the pharynx. Pieces *A* and *C* were used in the experiments as representing respectively the most anterior and the most posterior portion of the first or chief member or zooid in animals of this size (Child, '11*b*). In the intact animal the susceptibility of the region corresponding to piece *A* is very much greater than that of the region corresponding to piece *C*. Immediately after section the susceptibility of piece *A* shows either a slight increase or no marked change, while the susceptibility of *C* is increased to such a degree that it is equal to or even greater than that of *A* (Child, '14). The region *B* between *A* and *C* is intermediate both as regards original susceptibility and the changes following section and is not used in these experiments. The increase in susceptibility following section, which is most marked in the *C*-piece is

temporary and after six to eight hours is in course of disappearance and sooner or later (12 to 24 hours under ordinary conditions) the susceptibility of the piece becomes about the same as or a little less than that of the corresponding regions in the intact animal. These temporary changes in susceptibility indicate that the pieces have been stimulated by section, anterior pieces

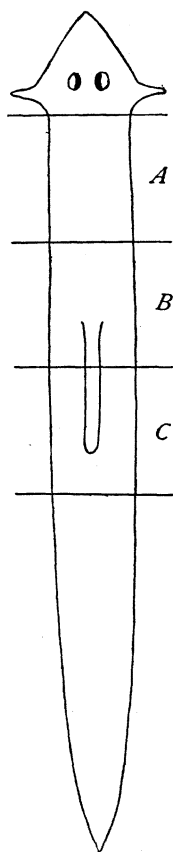


FIG. 1.

least, posterior pieces most, and that the condition of excitation disappears after a few hours. It has been suggested elsewhere (Child, '14) that the difference in the degree of stimulation in anterior and posterior pieces results from the greater dependence of the more posterior regions upon impulses from regions anterior to them, so that when the paths of these impulses are cut the condition of the more posterior levels is more affected than that of the anterior levels. This is in agreement with the observations of various investigators concerning the difference in intensity of reaction in many animals between levels anterior and those posterior to a transverse cut.

The region of the body posterior to *C* in Fig. 1 is not used in the tabulated experiments because it consists of one or more zooids indicated by differences in physiological condition (Child, '10, '11c). Since the number of these posterior zooids differs in different animals and no morphological boundaries are visible, pieces from the same levels of this region in different animals are not always strictly comparable physiologically and therefore merely complicate the experimental data.

Each experiment on CO_2 production in the pieces *A* and *C* consists essentially in determining the rate of change in pH , once as soon as possible after section and again several hours later, of one lot of *A*-pieces and one of *C*-pieces, consisting of thirty to fifty pieces each and of as nearly as possible equal weight, in equal volumes of indicator solution at a constant temperature

of 18° C. As soon as *pH* 7.3 is reached in the first determination, the tubes are opened, the pieces are returned to water and left undisturbed for the desired number of hours, *i.e.*, at least long enough as determined by the susceptibility method for disappearance in large measure of the temporary changes following section. At the end of this time the rate of *pH* change is again determined for each lot under as nearly as possible the same experimental conditions as the first determination.

Fifteen such experiments were performed before the technique used in the experiments recorded in Table I. was fully worked out. In eight of these fifteen experiments the *A*-pieces showed marked motor activity and a more rapid change in *pH* than the *C*-pieces immediately after section, in three there was either death of some of the pieces from drying or an error in weighing, and in four none of these sources of error was involved and the result were similar to those in Table I.

Table I. gives the data for thirteen experiments, in all of which the technique was as nearly as possible the same. The only feature of the table which requires explanation is the column "Time after section." The times given in this column are the times of sealing of the tubes containing the animals in indicator solution. The first time for each lot, "Immed.," *i.e.*, immediately, means simply that the *pH* determination was begun as soon as possible after the pieces were sectioned. Since it requires a half hour or more to cut lots consisting of forty to fifty *A* and *C*-pieces, the time between section and sealing may be as much as an hour for the pieces cut first and only a few minutes for those cut latest.

In eleven of the thirteen experiments recorded, 84 per cent., the CO₂ production of the *C*-pieces immediately after section is about equal to, or greater than that of the *A*-pieces. As regards the other two experiments, Nos. 7 and 8, in No. 7 three *C*-pieces died during the first determination, and in No. 8, motor activity occurred in the *A*-pieces, perhaps because of a slight rise in temperature of the water in which the tubes were kept. The second *pH* determination, begun at various lengths of time ranging from nine to forty-two hours after section, shows in every case a lower rate of CO₂ production in *C* than in *A*.

TABLE I.

A COMPARISON OF ANTERIOR AND POSTERIOR PIECES IMMEDIATELY AFTER SECTION AND A NUMBER OF HOURS LATER.

Expt. No.	No. of Pieces.	Wt. in Grams.	No. of Days Without Food.	Temperature in Degrees C.	pH Starting Point.	Time After Section in Hrs. and Min.	Time in Hrs and Min, to Reach pH 7.3.
1	A 37	0.023	11	17.5	7.8	immed. 22:30	5:40 5:40
	C 31	0.0231	11	17.5	7.8	immed. 22:30	5:25 5:55
2	A 49	0.0255	24	18	7.9	immed. 9:00	6:25 6:30
	C 45	0.0257	24	18	7.9	immed. 9:00	6:15 6:45
3	A 56	0.0245	21	18	7.82	immed. 19:40	5:18 6:05
	C 48	0.0243	21	18	7.82	immed. 19:40	5:05 6:23
4	A 20	0.0182	8	18	7.83	immed. 18:40	6:10 6:22
	C 23	0.0185	8	18	7.83	immed. 18:40	6:00 6:45
5	A 51	0.0208	9	18	7.83	immed. 20:15	5:30 5:35
	C 44	0.0207	9	18	7.83	immed. 20:15	5:05 5:55
6	A 37	0.0174	12	18	7.83	immed. 18:00	6:30 6:35
	C 42	0.0176	12	18	7.83	immed. 18:00	6:10 6:45
7 ¹	A 37	0.0143	13	18	7.83	immed. 21:30	7:20 7:45
	C 37	0.0142	13	18	7.83	immed. 21:30	7:35 8:00
8 ²	A 40	0.0216	7	18	7.83	immed. 17:45	4:50 5:05
	C 41	0.0213	7	18	7.83	immed. 17:45	4:55 5:10
9 ³	A 36	0.0238	8	18 18.5	7.83	immed. 19:00	4:50 4:38
	C 36	0.0239	8	18 18.5	7.83	immed. 19:00	4:35 4:50
10	A 37	0.0242	9	18	7.83	immed. 22:00	4:40 4:55
	C 38	0.0241	9	18	7.83	immed. 22:00	4:15 5:15
11	A 44	0.0242	7	18	7.95	immed. 19:00	5:30 5:35
	C 45	0.024	7	18	7.95	immed. 19:00	5:10 5:50
12	A 44	0.0306	8	18	7.95	immed. 42:00	4:45 4:40
	C 50	0.0305	8	18	7.95	immed. 42:00	4:15 4:50
13	A 29	0.021	13	18	7.8	immed. 25:00	4:05 4:10
	C 21	0.0208	13	18	7.8	immed. 25:00	3:50 4:25

As regards the two records for the same lot, the *A*-pieces show in general a slightly lower CO₂ production in the second period than in the first, or in some cases about the same. Experiment 9, however, shows a slightly higher rate in *A* during the second period, this being due probably to the slightly higher water temperature during the second period. In the *C*-pieces the rate is distinctly lower, in many cases much lower in the second period than in the first. Differences in time of five minutes between the records of two lots made at the same time mean only that the two were barely distinguishable as different. Differences of this magnitude between the two records of the same lot can mean no more than that the rate is essentially the same in the two periods. Differences of ten minutes or more are however, unquestionably significant and differences of much greater magnitude appear in the table.

The table shows then that immediately after section there is in some cases a slight temporary increase in CO₂ production in the *A*-pieces and a very marked increase in the *C*-pieces, that is to say the differences and changes in CO₂ production following section are in general parallel to the differences and changes in susceptibility. The objection may be raised that the differences in CO₂ production are less than would be expected from the stimulation by section, but when it is remembered that there is no reason to believe that the alimentary tract shares in this stimulation except locally at the cut surface and that the pieces do not undergo motor activity during the determination this objection has little weight. As a matter of fact the close parallelism between the data on CO₂ production and those on susceptibility indicates that even as regards the temporary changes following section of pieces, susceptibility is in some degree

¹ During the first determination in No. 7 three *C*-pieces died. When the determination was repeated the following day, two *C*-pieces, which had been cut with the others but not used before, were added to lot *C* and one piece was removed from lot *A* to make weights as nearly as possible equal. Only two extra worms had been sectioned, therefore three pieces could not be added to take the place of those dead.

² Slight motor activity occurred in the *A*-pieces during the first determination.

³ Here the temperature was half a degree higher in the second determination than in the first, and the *A*-pieces show a slightly increased rate in the second determination. Footnotes for Table 1.

a measure of physiological and particularly of respiratory condition.

CARBON DIOXIDE PRODUCTION AFTER REGENERATION.

In these experiments stocks of pieces of the size of *A*, *B*, *C*, Fig. 1, were cut from animals 16–18 mm. and allowed to undergo regeneration until the development of the new individuals was essentially complete, usually about two weeks. From these stocks the experimental lots were selected. These consisted only of normal individuals, or in some cases where the number of normal animals in the regenerated stock was not sufficient, mostly of normal with a few teratophthalmic individuals (Child, '11a) added. Since the pieces undergoing regulatory development cannot feed until they attain an advanced stage, the animals representing the condition before regulation, with which the regenerated individuals are to be compared, are kept without food for the same length of time as the pieces undergoing regeneration. Usually a stock of intact worms of the same size, 16–18 mm., as those from which the pieces were taken is isolated at

TABLE II.

A COMPARISON OF SMALL ANIMALS REGENERATED FROM PIECES WITH LARGE ANIMALS THAT HAVE NOT RECENTLY UNDERGONE REGENERATION OR FISSION, OF THE SAME SIZE AS THOSE FROM WHICH THE PIECES WERE TAKEN. BOTH UNFED. DETERMINATIONS OF pH AT +18° C.

Expt. No.	No. of Worms.	Wt. in Grams.	Period of Regeneration or Starvation in Days.	pH Starting Point.	Time in Hrs. and Min. to Reach pH 7.3.
1	3 large	0.0114	10	7.89	10:25
	16 regen.	0.0113	10	7.89	9:00
2	4 large	0.0163	11	7.89	8:55
	22 regen.	0.016	11	7.89	6:45
3	4 large	0.0174	10	7.89	8:00
	27 regen.	0.0176	10	7.89	6:15
4	4 large	0.0117	14	7.9	14:35
	19 regen.	0.0115	14	7.9	12:35
5	5 large	0.0205	14	7.9	9:35
	34 regen.	0.0204	14	7.9	6:50
6	3 large	0.0101	21	7.8	10:00
	26 regen.	0.099	7 starved 14 regen.	7.8	7:45
7	3 large	0.0109	20	7.8	8:30
	19 regen.	0.011	7 starved 13 regen.	7.8	7:50
8	3 large	0.0166	10	7.8	6:30
	28 regen.	0.0164	10	7.8	3:50

the same time the pieces are cut. This stock merely undergoes a slight degree of starvation, while the pieces undergo starvation for the same period and in addition the regulatory changes. This is the procedure in experiments 1-5 and 8 in Table II., but in experiments 6 and 7 the stock from which both pieces and whole animals were obtained was starved seven days before the pieces were cut.

Each experiment in Table II., includes one lot of worms about 5 mm. in length which have developed from pieces cut ten to fourteen days earlier ("regen." in table), and one lot of as nearly as possible the same weight of worms 16-18 mm. in length, the same size and from the same general stock as the worms from which the pieces were cut, and kept without food for the same length of time ("large" in table). In all cases the *pH* determinations are made before feeding is resumed.

Examination of the last column of the table shows that the rate of CO₂ production is much higher in the small regenerated, than in the large old animals, *i.e.*, the regulatory processes have been accompanied by an increase in rate of CO₂ production. Moreover the rate is in general higher in the regenerated animals

TABLE III.

A COMPARISON OF SMALL ANIMALS REGENERATED FROM PIECES WITH LARGE ANIMALS OF THE SAME SIZE AS THOSE FROM WHICH THE PIECES WERE TAKEN.

Fed three times. Determinations of *pH* at 18° C.

Expt. No.	No. of Worms.	Wt. in Grams.	<i>pH</i> Starting Point.	Time in Hours and Minutes to Reach <i>pH</i> 7.3.
1	3 large	0.0127	7.82	5:00
	10 regen.	0.0125	7.82	4:30
2	4 large	0.0193	7.82	3:45
	15 regen.	0.0191	7.82	2:55
3	4 large	0.0154	7.82	4:50
	13 regen.	0.0155	7.82	3:35
4	4 large	0.0135	7.9	7:15
	18 regen.	0.0134	7.9	5:50
5	5 large	0.0233	7.9	5:05
	28 regen.	0.023	7.9	3:45
6	2 large heads off	0.0155	7.8	8:15
	18 regen.	0.0113	7.8	4:25

than in the pieces of Table I. although the period without food is in most cases longer in the latter than in the former and CO₂ production decreases during the early stages of starvation.

Table III. records experiments similar to those of Table II.

except for the fact that both lots were fed with beef liver three times before weighing and pH determination, the first two feedings being on successive days, the third after an interval of one day. In the first five experiments of Table III. worms from the first five experiments of Table II. were used, but in smaller numbers because of the increased weights after feeding, particularly in the regenerated animals. In Table III., as in Table II. the "large" animals are those which have not undergone regeneration and represent as nearly as possible the animals from which the pieces were taken, and the "regenerated" animals are those which have developed from the pieces. Table III. agrees with earlier work (Child, '19a) in showing that the rate of change in pH is increased in all animals by feeding after a period of starvation, but it also shows that the difference in rate between the regenerated and the large animals persists after feeding. Here again the data on CO_2 production agree with the results of the susceptibility method (Child, '15, Chap. IV.). Data on oxygen consumption recently published by Allen ('19) and by Hyman ('19b) also agree with these results.

ADDITIONAL DATA.

Table IV. includes a number of miscellaneous experiments of some interest.

In the course of regulatory development an outgrowth of new tissue occurs at anterior and posterior ends of each piece. All the facts indicate that this tissue, which forms the new head and posterior end, is more or less embryonic in character when it arises and possesses, at least at first, a higher rate of metabolism than the remainder of the piece. In order to determine whether the higher rate of CO_2 production in regenerated animals is due solely to the more intense activity of this new tissue or whether the rate is also increased in other parts, the new heads and posterior ends were removed from regenerated animals leaving only the so-called old or less extremely altered tissue of the middle regions. Lots of such pieces were then compared with lots of equal weight of freshly cut *A*-pieces (Fig. 1) and of animals 16-18 mm. like those from which the pieces were taken, the heads being removed from these large animals in order to

make them more nearly comparable in condition with the *A*-pieces and the headless regenerated animals.

Experiment 1 of Table IV. includes one lot of each of the three groups and it is seen that the "old" parts of the regenerated

TABLE IV.

MISCELLANEOUS DATA.

Regenerated animals from which anterior and posterior new tissue has been removed, compared with *A*-pieces and with large headless animals: whole regenerated animals compared with *A*-pieces and with growing worms of same size from stock. Fed or unfed. Determinations of pH at 18° C.; pH at beginning of experiment 7.8.

Expt. No.	No. and Condition of Animals.	Wt. in Grams.	Nutrition.	Time in Hours and Minutes to Reach pH 7.3.
1	3 large, heads off.	0.093	Starved 19 days.	8:25
	12 <i>A</i> -pieces.	0.094	Starved 19 days.	7:15
	11 regen. new tissue off.	0.095	Starved 18 days.	6:25
2	9 <i>A</i> -pieces.	0.08	Fed three times.	8:15
	22 regen. new tissue off.	0.082	Fed three times.	7:30
3	3 large.	0.0111	Fed three times.	7:35
	15 regen. new tissue off.	0.0108	Fed three times.	6:05
4	22 <i>A</i> -pieces.	0.0167	Starved 10 days.	4:10
	28 regen.	0.0164	Starved 10 days.	3:50
5	11 small.	0.0096	Fed three times.	6:25
	18 regen.	0.0095	Fed three times.	5:25

animals show a higher rate of change than the *A*-pieces, while the headless large animals show the lowest rate of all.

In Experiment 2 the "old" parts of regenerated animals are compared with *A*-pieces, both lots being fed three times before sectioning. The result is the same as in experiment 1, the rate being distinctly higher in the parts of regenerated animals than in the *A*-pieces.

In experiment 3 the "old" parts of regenerated animals are compared with large old animals from which the heads have not been removed, both lots being fed three times after a starvation period of about two weeks. The result is the same as in Experiment 1, the parts of regenerated animals showing the higher rate.

Experiment 4 is a comparison of *A*-pieces with entire regenerated animals, *i.e.*, including the new heads and posterior

ends. Here the difference in rate is proportionally about the same as in Experiment 2, but somewhat less than in Experiment 1.

In Experiment 5 regenerated animals are compared with stock animals of slightly larger size (the smallest in the stock at the time) which were kept without food while the pieces were regenerating, both lots being fed three times before the experiment, and both consisting of entire animals. Here again the regenerated animals show a higher rate of CO_2 production than the slightly larger stock animals.

A few other experiments performed by one of us and in some cases also by students in the laboratory, are briefly mentioned here without tabulation of the data. It has been found, for example, that the degree of increase in both susceptibility and CO_2 production occurring in regeneration depends upon the degree of reorganization which occurs. Consequently the smaller the piece in relation to the size of the body from which it is taken, the greater the amount of increase in susceptibility and CO_2 . Similarly in natural fission the posterior piece is not only smaller than the anterior but develops a new head at the anterior end and a prepharyngeal and pharyngeal region by reorganization and redifferentiation within the piece, while the anterior fission piece develops merely a new posterior end. In the animal developed from the posterior piece susceptibility and CO_2 production show a marked increase while in the anterior animal the only marked change in susceptibility is a slight increase in the posterior region, where reorganization and growth have occurred and the increase in CO_2 production is either slight or inappreciable. Allen ('19) has recently recorded somewhat similar results as regards oxygen consumption, an increase occurring in the posterior, but not in the anterior product of fission.

It has also been determined by one of us that susceptibility to lack of oxygen increases during the development of a new individual from a piece, the susceptibility of the new individual about two weeks after section of the piece, being distinctly higher than that of well fed animals of the same size and about the same as, or slightly than that of animals of the same size, kept without food for the same length of time as the regenerating

pieces. In other words, the new individuals developed from pieces show a susceptibility to lack of oxygen equal to or greater than that of much smaller younger animals than those from which the pieces were taken. These observations concern primarily the susceptibility of ectoderm and body wall.

CONCLUSION.

These experiments constitute a new test of the validity of the susceptibility method as a rough comparative means of determining physiological or metabolic condition and at every point the differences and changes in susceptibility, as determined by KNC and in many cases by various other agents also, are paralleled by differences and changes in rate of CO₂ production. Even the temporary stimulation of the pieces after section, which is slight or absent in the *A*-pieces and very marked in the *C*-pieces, appears in the data on CO₂ production and the increase in rate, at least of respiratory metabolism associated with regulation, is evident in the marked increase in rate of CO₂ production even in the "old" parts of the regenerated animal. This work may perhaps be regarded as in some respects the most delicate test of the relation between susceptibility and respiration which has been made up to the present.

As has been repeatedly stated, the susceptibility method is not an exact quantitative method, but a rather crude means of indicating differences of some sort in physiological condition and the differential susceptibility of different regions of the same individual affords a means of modifying and controlling various developmental and other processes. The facts at hand concerning susceptibility, *e.g.*, the lack of specificity, the close relation between susceptibility and physiological activity in development, growth and function as well as the positive evidence already obtained concerning the parallelism between susceptibility, oxygen consumption and CO₂ production indicate very clearly that a more or less definite relation exists between the susceptibility of living protoplasm, to at least many external agents and conditions within certain ranges of concentration or intensity, and the rate or intensity of certain fundamental physiological processes, particularly those which liberate energy.

This is all that is meant when susceptibility is interpreted in terms of metabolism or oxidation and the exact nature, degree and extent of this relation of course remains to be determined. This interpretation does not involve the assumption that susceptibility must always be parallel or even proportional to total oxidation or even to total oxygen consumption or CO_2 production.

The relation between susceptibility and oxidation is undoubtedly indirect in at least most cases and it is conceivable that susceptibility may be related only or primarily to certain oxidative reactions or to conditions associated with them. Moreover, it is certain that in many cases susceptibility as determined by death and disintegration is dependent primarily upon conditions or reactions in particular regions of the body, *e.g.*, in ciliate infusoria the ectoplasm, in *Planaria* the ectoderm and body-wall. Moreover, as many investigators have pointed out, it is by no means certain that oxygen consumption and CO_2 production are exact quantitative measures of oxidation at any given time. It is to be expected that susceptibility will not always be proportional to total oxygen consumption or CO_2 production, but even then susceptibility may prove in the long run to be a better indicator or comparative measure of physiological condition than the respiratory data.

From what has been said above and in earlier papers (*e.g.*, Child, '19c) it is evident that the criticisms of the susceptibility method recently advanced by Lund ('18*a, b*) and Allen ('18, '19) need no discussion here, since they are largely beside the point and result from failure to grasp the conception of susceptibility, which has developed from many different lines of investigation, not from one alone. Even if we grant the correctness of certain of their conclusions from experimental data which are or appear at present to be in conflict with conclusions reached in this laboratory (Child, '19*a, b, c*, Hyman, '19*a, b*) on the basis of more extensive investigation, with more satisfactory technique and several different methods instead of one they do not constitute adequate grounds for denying the physiological significance of susceptibility, but rather merely a starting point for the further analysis of the particular cases in question.

As regards the real significance of susceptibility, it makes

little difference whether or not it shall be found to run exactly parallel to the other indices of total respiration in any particular case. It cannot, however be denied that a wide range of facts determined by many different lines of investigation do indicate clearly the existence of a more or less definite relation between susceptibility and oxidation in at least many cases, and it is of interest to determine range, degree and nature of this relation. The present paper like several others which have recently appeared from this laboratory is a contribution to this problem, but it must be remembered that data such as these are not the only criteria of the physiological significance of susceptibility and its relation to the energy-liberating reactions in the metabolic complex.

SUMMARY.

1. The colorimetric estimation of CO₂ production shows that the changes in CO₂ production following section in pieces of the body of *Planaria dorotocephala* run parallel with changes in susceptibility. Immediately following section CO₂ production is markedly increased in pieces cut from near the mouth region, while in pieces from regions near the head it is only slightly if at all increased. These changes are temporary excitations following section and disappear after a number of hours.

2. The development of a new individual from a piece is accompanied by a very considerable increase in CO₂ production which involves not only the new outgrowths at the two ends of the new animal but the "old" parts as well. This increase in CO₂ production is found both before and after feeding is resumed following the development of the piece. In these respects also the changes in CO₂ production parallel changes in susceptibility, both series of data indicating that the animal developing from an isolated piece becomes in the course of this development, physiologically younger than the animal from which the piece originated.

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